Epidemiology and Global Health

Global risk mapping of highly pathogenic avian influenza H5N1 and H5Nx in the light of epidemic episodes occurring from 2020 onward

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eLife Assessment

The authors have undertaken a **useful** study to update an existing niche model of highly pathogenic avian influenza. However, there are issues regarding the conceptualisation of the ecological niche of highly pathogenic avian influenza transmission that the modelling aims to capture, raising concerns about the strength of evidence used to support the findings. There are a number of modelling assumptions that are **incompletely** justified. Combined with shortcomings in the communication, this dilutes the strength of the key findings of this work.

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Abstract

Avian influenza (AI) is a highly contagious viral disease affecting poultry and wild water birds, posing significant global challenges due to its high mortality rates and economic impacts. Highly pathogenic avian influenza (HPAI) outbreaks, particularly those caused by H5N1 and its variants, have surged since their first occurrence in 1959. The HPAI H5N1 clade 2.3.4.4b viruses have notably expanded its geographical reach, affecting numerous countries, diverse avian species, and now wild and domestic mammals. Using an ecological niche modelling approach, this study aims to elucidate the environmental factors associated with the increased HPAI H5 cases since 2020, investigate potential shifts in ecological niches, and predict new areas suitable for local viral circulation. Focusing on H5N1 and H5Nx strains, we have developed ecological niche models for HPAI case in both wild and domestic birds while considering two distinct periods: 2015-2020 and 2020-2022. Key environmental predictors include chicken and duck population density, human density, distance to water bodies, and

several land cover variables. Post-2020, we observe a notable increase in the relative importance of some of these predictors, such as intensive chicken population density and cultivated vegetation. The resulting risk maps reveal notable ecological suitability for local HPAI H5 circulation in Europe, Asia, as well as North and South America, with notable expansions of the areas at risk post-2020. The spatial distribution of HPAI H5 occurrences in wild birds appears to be primarily influenced by urban areas and open water regions. Overall, global risk maps derived from our models identify regions at risk where surveillance and control measures should be prioritised. Finally, our analyses also highlight a shift in the diversity of species affected by HPAI outbreaks, with a higher variety of avian species, particularly sea birds, being impacted post-2020. This increased diversity suggests that ecological shifts in HPAI H5 circulation may be accompanied by a broader range of susceptible species. Overall, these results further contribute to the understanding of HPAI epidemiology.

Introduction

Since 2020, an increase in both H5Nx and H5N1 cases has been observed (**Fig. 1**). A variant of the Gs/Gd H5N1 viruses belonging to the H5 clade 2.3.4.4b has led to an unprecedented number of deaths in wild birds and poultry in many countries in Africa, Asia, and Europe (Huang et al., 2023). In 2021, the virus spread to North America, and in 2022, to Central and South America (FAO, WHO and WOAH situation analysis, July 12, 2023) spilling over into poultry farms and infecting an alarming number of wild terrestrial, marine and domestic mammals (Elsmo et al., 2023; Leguia et al., 2023; Neumann and Kawaoka, 2024; Peacock et al., 2024; Plaza et al., 2024); marking an unprecedented expansion in the geography and impact of HPAI. In 2022, 67 countries across five continents reported H5N1 HPAI outbreaks in poultry and wild birds to WOAH, resulting in over 131 million domestic poultry deaths or cullings (WOAH, 2023). In 2023, another 14 countries, mostly in the Americas, reported outbreaks. Several mass death events in wild birds were caused by influenza A(H5N1) clade 2.3.4.4b viruses (Klaassen and Wille, 2023; WOAH, 2023).

In that epidemiological context, understanding the environmental factors associated with the risk of HPAI circulation remains crucial. Extensive exploration of risk factors for HPAI presence, spread, and persistence has been conducted in various countries and regions. These studies have identified domestic waterfowl, several anthropogenic variables (human population density, distance to roads) and indicators of water presence as important factors for risk of H5N1 local circulation (Gilbert and Pfeiffer, 2012). More recent work on global suitability for HPAI have pinpointed that host-related variables such as poultry density are the strongest contributors to HPAI persistence (Dhingra et al., 2016; Gierak and Śmietanka, 2021; Martin et al., 2011).

In addition, poultry intensification, international poultry trade, live bird markets, and wild bird migratory routes have been recognised as playing a key role in the transmission and spread of HPAI (Vandegrift et al., 2010). Lastly, evidence of climate change impacting the dynamics of HPAI has also been discussed (Prosser et al., 2023). For example, changes in rainfall alter the distribution, abundance, and quality of wetlands and can impact waterfowl populations (Forcey et al., 2007; Gilbert et al., 2008; Vandegrift et al., 2010). However, recent outbreaks raise questions on the ability of previous models to assess HPAI H5 ecological suitability.

Understanding the interplay between environmental variables and avian influenza becomes increasingly crucial to have an in-depth understanding of the risk factors that govern AIVs circulation and spread. Following a previous study dedicated to the ecological niche of H5NX and H5N1 (Dhingra et al., 2016), we aimed to address the following questions: (i) what factors can explain the observed increase in H5Nx and H5N1 cases since 2020? (ii) Do we observe a change or an extension of the ecological niche beyond its traditional spatial coverage? (iii) are we able to

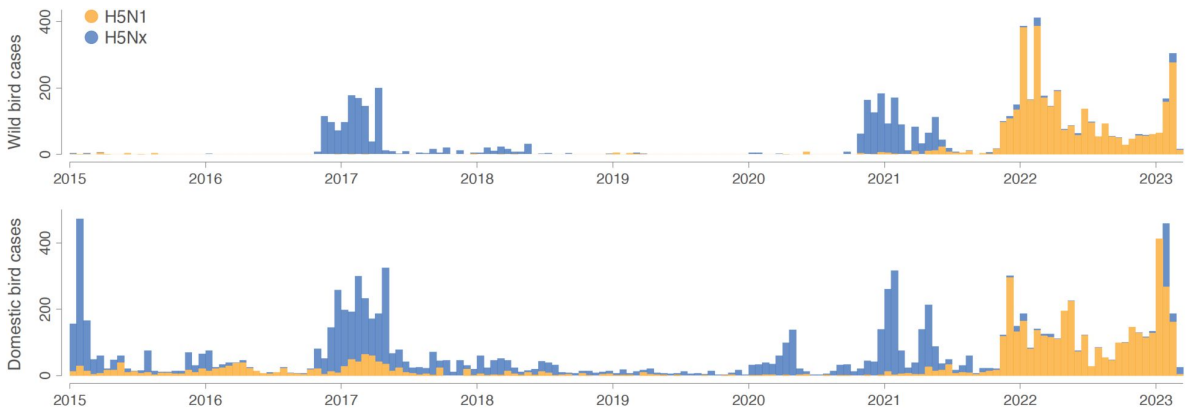

Figure 1

Epidemic curves for both wild and domestic bird cases.

Orange and blue histograms report the weekly number of H5N1 cases and the weekly number of H5Nx cases, respectively.

predict the areas at risk based on ecological niche models trained on occurrence data before 2020? To this end, we used HPAI H5N1 and H5Nx occurrence data collected before 2020 to train ecological niche models and tested whether these models could accurately predict areas ecologically suitable for HPAI circulation post-2020. Specifically, we employed a boosted regression trees (BRT) approach to estimate the HPAI ecological suitability given local environmental conditions. We also computed the diversity indices of wild bird species involved in HPAI occurrences, helping to understand how shifts in species diversity might correspond with the environmental factors considered in our ecological niche modelling analyses. Our results provide a better understanding of HPAI dynamics by identifying key environmental factors driving the increase in H5Nx and H5N1 cases in poultry and wild birds, investigating potential shifts in their ecological niches, and improving the prediction of at-risk areas. This knowledge might aid policymakers, epidemiologists, and wildlife conservationists in better targeting surveillance and control measures.

Results

In this study, we apply an ecological niche modelling approach using the boosted regression trees (BRT) method to evaluate the suitability of environments for H5Nx and H5N1 HPAI viruses in wild and domestic birds across two periods: 2015-2020 and 2020-2022. Specifically, we trained distinct ecological niche models for each combination of species (wild or domestic birds), virus strain (H5Nx or H5N1), and study period. In addition to evaluating each model separately, models trained on data from the 2015-2020 period were tested to predict occurrences in the 2020-2022 period, allowing us to evaluate the potential of pre-2020 models to predict a more recent distribution of H5N1 and H5Nx cases.

We assess the influence of a broad range of spatial predictors — including anthropogenic, topographical, land cover, eco-climatic, and poultry density — on virus occurrences (**Fig. S1**). We use occurrence records from the EMPRES-i database, and sample pseudo-absence points to account for the lack of virus detection data in some areas. These pseudo-absence points were distributed based on human population density, with more pseudo-absence points sampled in areas of higher population to reflect the greater surveillance efforts in those regions (**Fig. S2**).

The accuracy of the models was validated through three types of cross-validation: (i) a standard cross-validation with a random and stratified divide between training and validation sets, (ii) a spatial cross-validation based on the approach used by Dhingra and colleagues (2016), and that consists in clustering presence and pseudo-absence points into folds using reference presence points, and (iii) a second spatial cross-validation based on a blocks generation technique (Valavi et al., 2019). Models using spatial cross-validation techniques are less affected by spatial autocorrelation, as shown by higher spatial sorting bias (SSB) values (**Fig. S3**). Of the seven datasets evaluated, four show better performance with the reference points-based spatial cross-validation method, suggesting it is less impacted by spatial autocorrelation compared to the block generation technique. Consequently, we have proceeded with the reference points approach for consistency with Dhingra et al. (2016).

Using pre-2020 data, our models demonstrate a relatively robust capability to predict the distribution of post-2020 occurrence data: the Area Under the Curve (AUC) values for ecological niche models trained occurrence data in domestic birds before 2020 range between 0.74 and 0.77 when evaluated with occurrence data in domestic birds after 2020, indicating good predictability. In comparison, ecological niche models trained on occurrence data in domestic birds after 2020 display slightly higher predictive performances when evaluated with the same data, with AUC values ranging between 0.78 and 0.83 (**Table S1**). These findings underscore the pre-2020 models' effectiveness in forecasting the recent geographic distribution of ecological suitability for H5Nx and H5N1 occurrences.

Previous literature reviews (Gilbert and Pfeiffer, 2012; Dhingra et al., 2016) have summarised the predictor variables commonly linked to occurrences of HPAI. As detailed in the Materials and Methods section and in the table in Supplementary Information Resources S1, we explored four sets of environmental variables considered by Dhingra and colleagues (Dhingra *et al*. 2016): host variables (set 1), land cover variables (set 2), eco-climatic variables (set 3), and a risk-based selection of variables performed by Dhingra and colleagues (set 4). Set 3 shows the lowest predictive performance for both domestic and wild bird cases during the two periods and, in contrast, sets 2 and 4 demonstrate the highest predictive accuracy for wild and domestic birds, respectively (**Fig. S3**). Set 4 includes a combination of host variables with cultivated and managed vegetation, open water areas, distance to water, and the annual mean of land surface temperature. For domestic birds, set 1, which includes host variables, shows high predictive performance before 2020, but its performance decreases after 2020 in favour of set 4. The ecological niche models trained on wild and domestic bird cases, respectively with the sets 2 and 4, are associated with AUC values exceeding 0.77, indicating relatively good predictions of H5 occurrences with these sets of environmental variables.

Figure 2 displays the response curves of the most important variables with their respective relative influence (RI) (**Table S2**). For the H5N1 and H5NX ecological niche models for domestic birds, densities of intensive chicken population, duck population, and human population emerge as significant predictors, each with RI values exceeding 5%. Notably, there is a sharp increase in the RI for H5N1 in areas with high densities of intensive chicken populations — from 8.5% to 30.4% since 2020. Similarly, the RI of cultivated and managed vegetation has doubled for both strains post-2020. Moreover, the response curves associated with these predictors show a positive correlation, indicating that higher values of these predictors are linked with an increased likelihood of HPAI occurrences given local environmental conditions. This result indicates a trend towards increased HPAI susceptibility in environments characterised by intensive agricultural and vegetation management practices.

In contrast, eco-climatic factors such as land surface temperature and precipitation (set 3) showed only moderate influence. We also observe a decrease in the importance of duck population density for both subtypes after 2020, possibly due to the increasing diversity of bird species involved in the transmission dynamics of H5N1 and H5NX (**Fig. S4**). These findings highlight the crucial role of anthropogenic and host-related variables in accurately predicting HPAI occurrences.

In the ecological niche models trained for wild bird cases, urban and built-up areas are associated with H5N1 and H5Nx outbreaks (**Fig. 2**), and have the highest relative importance (RI) prior to 2020. Specifically, the RI values were 54.5% for the period before 2020 and decreased to 39.3% for predictions extending beyond 2020. This decline in RI values may indicate a reduced bias in observation data; typically, dead wild birds are more frequently found near human-populated areas, which could influence earlier data sets. After 2020, we observe an increase in the importance of habitats, such as deciduous broadleaf trees (6.1%), mixed and other tree regions (11.4%), and herbaceous vegetation for H5Nx (9.5%). However, as illustrated by the response curves (**Fig. 2**), the presence of H5N1 and H5Nx in wild birds declines in areas with greater tree cover, while it increases in regions dominated by herbaceous vegetation. Open water areas consistently show high RI values across all time periods and virus strains, particularly for H5Nx before 2020 (25.5%) and H5N1 after 2020 (22.0%), highlighting their significant role in the ecological models of HPAI outbreaks.

The risk maps generated show the predicted ecological suitability for H5N1 and H5NX avian influenza viruses across two time periods, 2015-2020 and 2020-2022 (**Fig. 3**), and are also accessible on the following link for a dynamic visualisation of the results: *https://mood-platform.avia-gis.com/core*. These maps reveal both broad regional risks and pinpointing specific locales of heightened susceptibility, especially in Europe and Asia (India and China). Our models highlight extensive spread of H5N1 and H5Nx HPAI among domestic birds in South Korea, Japan, Singapore,

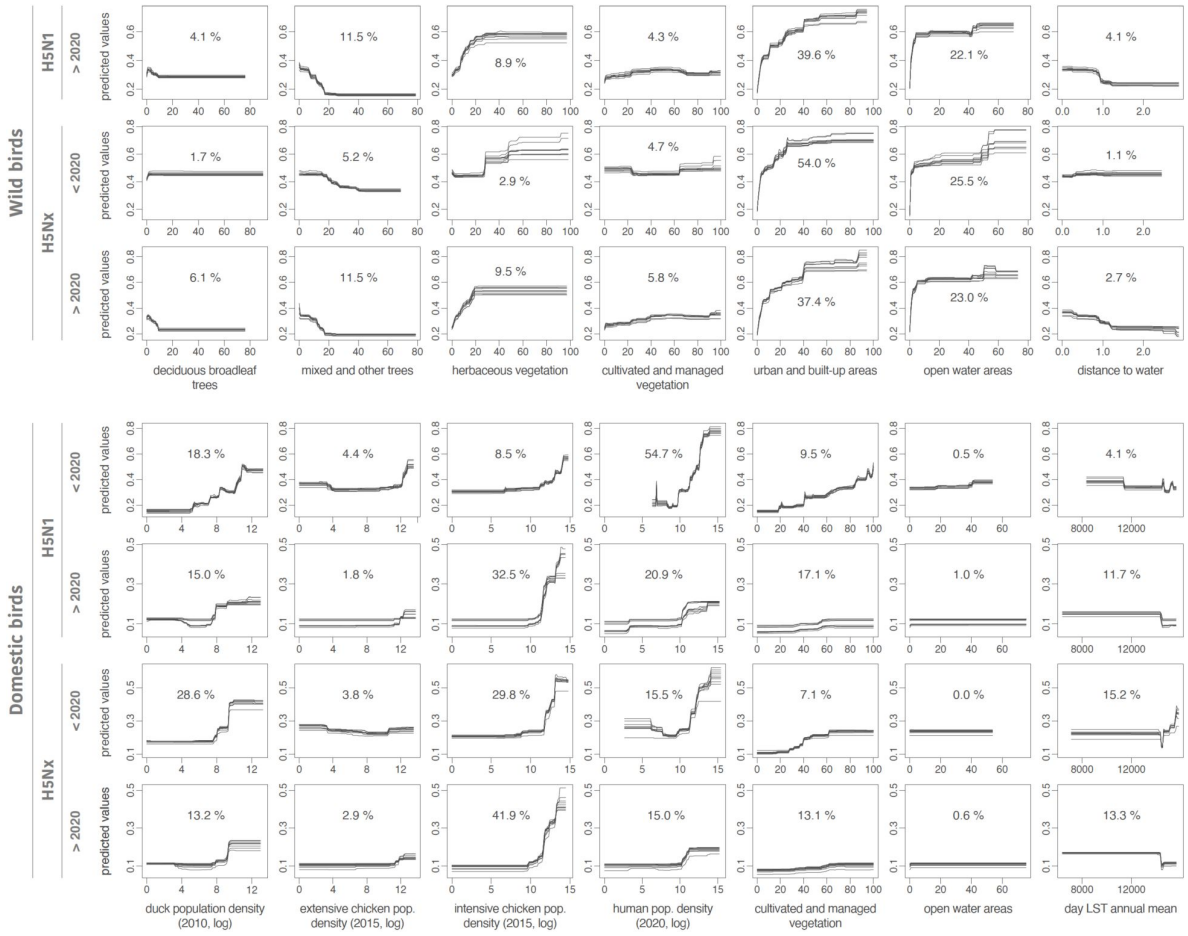

Figure 2

Response curves associated with the environmental variables included in the ecological niche models.

For the ecological niche models trained on wild bird infection records, we here only display the response curves estimated for the environmental variables associated with an averaged relative influence (RI) >4% for at least one of the considered occurrence datasets (thus not reporting the response curves obtained for the following variables: evergreen deciduous needleleaf trees, evergreen broadleaf trees, shrublands, and regularly flooded vegetation). Each curve was retrieved from a distinct boosted regression tree (BRT) model trained for a specific dataset of occurrence data. We also report the averaged RI (in %) of each environmental variable in the respective ecological models trained on a specific dataset of occurrence data (see **Table S1** for the complete list of RI estimates along with their first and third quartiles). Due to lack of data, the model was not trained for H5N1 in wild birds before 2020.

Malaysia, Vietnam, Cambodia, Thailand, and the Philippines, as well as in European countries such as the United Kingdom, France, the Netherlands, Germany, Italy, Ukraine, and Poland. Additionally, several areas in African nations, including Nigeria and South Africa, are identified as suitable environments for both H5N1 and H5Nx occurrence in scenarios before and after 2020. Regions in North America and South America — including Bolivia, Brazil, Colombia, Ecuador, Chile, Peru, and Venezuela — demonstrate ecological suitability for H5Nx and H5N1, with the period after 2020 showing a marked increase in the risk of local H5N1 circulation.

Our findings remain overall consistent with the ones previously reported by Dhingra and colleagues (Dhingra et al., 2016), who used data from January 2004 to March 2015 for domestic poultry, even if we can note some differences: North America, West Africa, eastern Europe and Bangladesh have a higher risk of H5 infection before 2016, while our maps mainly highlight regions in China, South-East Asia, and Europe (**Fig. S5**). In India, both analyses consistently pinpoint strong risk areas for H5N1 and H5Nx, though our data suggests these areas have higher ecological suitability. Similar to results reported by Dhingra and colleagues, we observe an increase in the ecological suitability estimated for local H5N1 circulation in South America's domestic bird populations post-2020. Finally, in North America, while Dhingra and colleagues predicted higher risk areas for H5Nx, our findings highlight similar regions but with lower ecological suitability.

For H5Nx and H5N1 cases in wild birds, the estimated ecological niches are mostly related to environmental factors such as open water and distance to water, which result in ecological suitability hotspots estimated near coasts and main rivers. For both H5Nx and H5N1, however, isolated areas on the risk map should be interpreted with caution. These isolated areas may result from sparse data, model limitations, or local environmental conditions that may not accurately reflect true ecological suitability. The risk maps reveal that high-risk areas have expanded after 2020. North America appears to be more susceptible to H5Nx in particular near the Great Lakes region. This expansion is evident in Russia, South America, and North America aligning with major bird migration routes. Notably, while H5Nx has never been reported in Australia, the models indicate potential ecological suitability there as well. Additionally, regions in China, India, and Europe also display expanded areas of ecological suitability.

In **Table 1**, we report the estimation of avian species diversity indices for species involved in HPAI transmission for the pre-2020 and post-2020 periods. We observe variations between these two periods both in the overall bird population and within specific wild bird species groups, including marine species. For all birds, the Shannon index increased from 4.23 before 2020 to 5.03 after 2020, indicating a more diverse infected bird population in the latter period. The Simpson index also rose from 0.97 to 0.98, suggesting a slightly higher concentration of certain species post-2020. Sea birds exhibited a similar upward trend in diversity post-2020, with the Shannon index increasing from 2.19 to 2.26. This rise in Shannon index for sea birds suggests a broadening of species diversity within this category from 2020 onwards.

When considering birds affected by the H5N1 strain of HPAI, there is a notable increase in the Shannon index from 2.00 before 2020 to 2.95 after 2020, accompanied by a rise in the Simpson index from 0.83 to 0.91. These increases could be indicative of greater diversity and a more even distribution of species affected by H5N1 after 2020. In contrast, birds not affected by the H5N1 strain showed a slight decrease in diversity post-2020. The Shannon index decreases from 3.42 to 3.26, and the Simpson index decreases from 0.95 to 0.92. Given that HPAI particularly affected sea birds >2020, we further explore the yearly distribution of sea bird families. As depicted in **Fig. S4**, sea birds belonging to the families Laridae, Sulidae, Ardeidae, Pelecanidae, Spheniscidae, Alcidae, Phalacrocoracidae were more prevalent >2020 in comparison to <2020. Especially, the families Laridae and Sulidae have a special increased prevalence from 2021 onwards.

Figure 3

Ecological niche suitability estimated for H5N1 and H5Nx.

We estimated the ecological suitability for two different time periods (2015-2020 and 2020-2022) and for both wild and domestic bird populations. Dynamic visualisation of the results are available here: *https://mood-platform.avia-gis.com/core*.

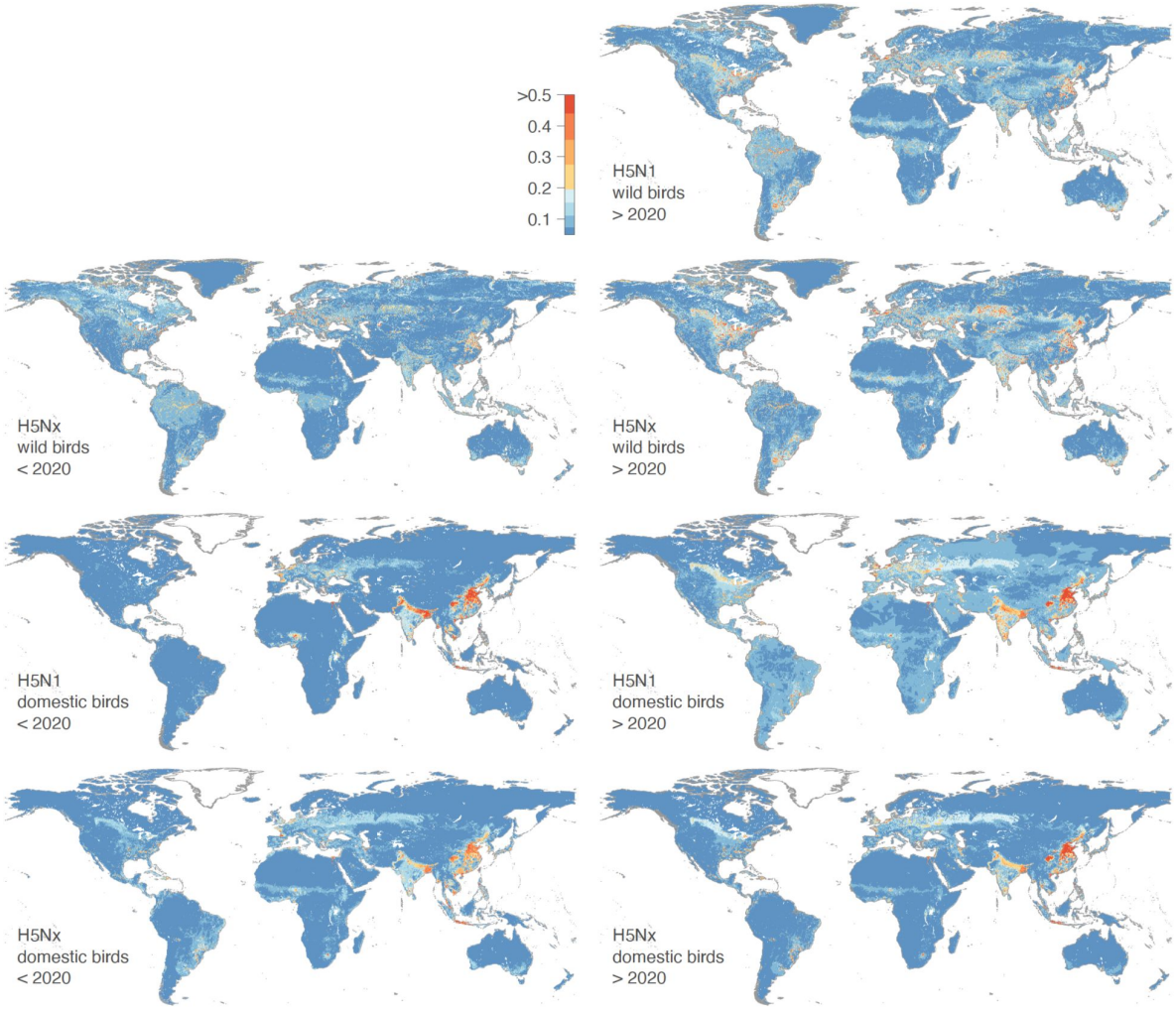

Table 1.

Bird diversity indices before and after 2020.

This table presents the Shannon and Simpson diversity indices for various bird groups, comparing values before and after the year 2020. The indices are provided for all birds, sea birds, wild birds, domestic birds, and birds affected by H5N1 and non-H5N1 strains.

	All birds		Sea birds		All birds H5N1		All birds non-H5N1		Wild birds		Domestic birds	
	Shannon	*Simpson*	*Shannon*	*Simpson*	*Shannon*	*Simpson*	*Shannon*	*Simpson*	*Shannon*	*Simpson*	*Shannon*	*Simpson*
< 2020	4.227	0.971	2.187	0.843	2.003	0.833	3.415	0.945	3.072	0.832	3.919	0.966
> 2020	5.033	0.982	2.255	0.831	2.952	0.907	3.254	0.914	5.066	0.983	2.967	0.897

Discussion

Using an ecological niche modelling approach, our study presents an in-depth analysis of the ecological suitability for the risk of local circulation of H5Nx and H5N1 HPAI viruses across different temporal and ecological contexts. By conducting boosted regression tree (BRT) analyses and incorporating a diverse set of spatial predictors, our study provides insights into the environmental and anthropogenic factors influencing the distribution of these viruses in both wild and domestic bird populations. For domestic birds, our results concur with the those previously reported by Dhingra and colleagues (Dhingra et al., 2016), indicating that ecological niche models incorporating host variables (chicken and duck population densities) outperform those based on land-use or eco-climatic factors in terms of predictive performance. Contrariwise, in our analysis of H5N1 and H5Nx HPAI in wild birds, the most significant predictors were anthropogenic factors, notably urban areas and distance to open water. However, observation biases might affect these findings since outbreaks in wild birds are commonly reported near human populations.

In our analyses, intensive chicken population density emerges as a significant predictor, underscoring the influence of poultry farming practices on HPAI spread (Dhingra et al., 2016; Vincenti-Gonzalez et al., 2024) and revealing a complex interplay between ecological niches and the anthropogenic landscape. Similarly to the results of Dhingra and colleagues (Dhingra et al., 2016), land-use and climatic predictors do not play an important role in the niche ecological models, even for wild birds. Additionally, we found that while duck population density is a significant predictor for HPAI occurrence in domestic birds before 2020, its importance has decreased post-2020, particularly within the H5Nx ecological niche model. This observation is particularly noteworthy, as ducks have historically been closely linked to areas of persistence and evolution of H5N1 HPAI (Gilbert and Pfeiffer, 2012). However, our findings, in line with earlier studies such as the one of Dhingra and colleagues (Dhingra et al., 2016), highlight a significant shift in the epidemiological dynamics of HPAI. Specifically, while the association between the H5Nx clade 2.3.4.4 and ducks has for instance been documented in South Korea (Hill et al., 2015), it is becoming increasingly evident that HPAI is now more strongly associated with high-density chicken farming and anthropogenic factors. This shift suggests that the virus could now be circulating more among farms (farm to farm transmission) (FAO, 2023), with fewer introductions by wild birds — particularly wild ducks, which have previously acted as “Trojan horses” for H5N1 HPAI (Kim et al., 2014). Consequently, we could be witnessing a shift from the traditional duck/rice ecosystems towards a scenario where intensive chicken farming and a wider diversity of wild birds play a more prominent role in the transmission dynamics of HPAI. Therefore, monitoring areas with high intensive chicken densities and regular surveillance of wild birds remains crucial for the early detection and management of HPAI outbreaks.

A difference between the ecological niche models previously trained by Dhingra and colleagues (Dhingra et al., 2016) and our models is the pronounced increase in ecological suitability for H5N1 in domestic bird populations estimated in India and China. More generally, our models highlight the extensive spread of H5N1 and H5Nx HPAI among domestic birds in Asia previously identified as “reassortment sink areas” (Dhingra et al., 2018). Reassortment refers to the process where influenza viruses exchange genetic material, potentially leading to new, more virulent strains. The extent of virus suitability in Asia highlights a potential change in the dynamics of virus spread, possibly driven by intensified poultry farming practices and increased human-wildlife interactions in these regions (Bahl et al., 2016; Gilbert et al., 2017).

The recent outbreaks of infection in cattle within the United States have been partially delineated by our HPAI risk mapping approach, highlighting major hotspots predominantly in the Great Lakes region, including Michigan. From March to July 2024, approximately 26 herds in Michigan

were affected (U.S. Department of Agriculture, 2024). Additionally, our risk maps identify localised hotspots around urban centres in Colorado and Texas, where herds have also been infected. These outbreaks appear to stem from a single introduction event in Texas by wild birds (Worobey et al., 2024a, 2024b). This trend may be indicative of an increasing risk of future introductions from wild birds, as we observed a significant rise in the diversity of infected bird species after 2020, particularly among sea birds.

The latest strain of H5N1 (H5 clade 2.3.4.4b), believed to have emerged in 2014, shows adaptations for infecting a wide range of wild birds and mammals (Caliendo et al., 2022; Graziosi et al., 2024). By 2020, its spread among wild birds was three times faster than in farmed poultry due to mutations enabling the virus to infect diverse species (CDC, 2024; Xie et al., 2023). Unlike previous epidemic waves in Europe, outbreaks in the last years have also continued through summer, confirming that the virus's current circulation is no longer seasonal but nesting in the wild bird population (Sciensano, 2024). Therefore, the virus is able to infect a wider range of species than previous forms, as it circulates all seasons (Sacristán et al., 2024). The densely packed colonies of sea birds have also facilitated the rapid virus spread, resulting in high mortality in these sea bird colonies (Boulinier, 2023; Bregnballe et al., 2024). For instance, the Netherlands lost up to 80% of its Sandwich Terns in weeks, and the UK saw significant outbreaks, especially on Scottish islands (Knief et al., 2024). In South America, similar trends have been observed, with notable die-offs in seabird populations along coastal regions, particularly impacting species such as penguins, pelicans and cormorants (Leguia et al., 2023). Since then, in early 2023, the Peruvian outbreak had spread to marine mammals, particularly affecting the South American sea lion, which also began to experience a mass die-off (Campagna et al., 2024; Leguia et al., 2023).

The increase in the number of wild bird species being infected by HPAI, particularly H5N1, could be attributed to several factors such as viral evolution, increased interaction between domestic and wild birds, climate and land-use changes, among others. Regarding land-use changes, large parts of Europe have become increasingly fragmented due to the expansion of urban and transport infrastructure (European Environment Agency (EEA), 2022). This habitat loss and fragmentation can significantly impact water availability and trophic resources (Santos-Tovar et al., 2024), which could in turn aid the spread and transmission of avian influenza viruses due to relocation of birds and outbreaks (Yin et al., 2022). Climate change has also been reported as a factor that could affect wild bird distribution in different ways (Gilbert et al., 2008). As global climate conditions change, avian migratory patterns and routes are also changing (Hitch and Leberg, 2007; Van Doren, 2022). Furthermore, higher temperatures and extreme weather have resulted in large-scale population shifts in a range of temperate species (Dezfuli et al., 2022). Overall, these elements therefore raise the question of the role of climate and land-use changes in the recent shifting epidemiology of HPAI in wild birds.

The increasing diversity of infected bird species is part of a broader resurgence of H5N1 globally. Phylogenetic analysis from Europe revealed significant genetic diversity in HPAI-H5N1 viruses since October 2020, likely due to multiple reassortment events with LPAI viruses. Notable introductions from Russia to Europe were observed through autumn migrations of wild birds (ECDC, 2021). Additionally, the transatlantic transport of HPAI H5N1, documented in Canada in December 2021, suggests the virus can spread across continents, a route previously seen only with LPAI viruses (Caliendo et al., 2022; Dusek et al., 2014; Huang et al., 2014). In November 2022, H5N1 (clade 2.3.4.4b) was introduced into South America, presumably through the movements of migratory wild birds travelling south during the boreal winter. The virus spread rapidly and caused significant mortality in wild birds and marine mammals across multiple countries (Leguia et al., 2023; (Banyard et al., 2023; Campagna et al., 2024; Gamarra-Toledo et al., 2023; WOAH, 2024).

Despite the robustness of our ecological niche models, some limitations must be acknowledged. Firstly, the accuracy of our models is inherently dependent on the quality and completeness of the occurrence data used, which is influenced by the different reporting systems in place in different countries. Secondly, the definitions of key explanatory variables, such as intensive versus extensive production, may vary across regions, adding complexity to global predictions. To address these limitations, future work could focus on developing country-level or regional models that incorporate localised data and context-specific definitions. Thirdly, underreporting and biases in outbreak reports, particularly in wild bird populations, can skew model predictions and underestimate areas at risk. Finally, another limitation of our model for wild bird populations is the lack of incorporation of migratory patterns and behaviours. Migratory birds have complex movement patterns influenced by seasonal changes, food availability, and habitat conditions. Our models, using static spatial predictors, do not capture these dynamic routes and stopover sites, leading to potential inaccuracies in predicting areas at risk for HPAI spread. In future work, the integration of dynamic migratory data and environmental changes into our ecological niche models could provide a more comprehensive and accurate risk assessment for HPAI spread among wild bird populations.

Despite these limitations, our study provides significant contributions to the understanding of H5N1 and H5Nx HPAI virus dynamics. By integrating a wide range of spatial predictors and using ecological niche modelling techniques, we provide a quantitative analysis on the factors driving virus distribution. Overall, our study underscores the importance of continuous surveillance and the need for adaptive management strategies to address the evolving threat of HPAI viruses globally.

Materials and Methods

HPAI occurrence data acquisition

The dataset containing highly pathogenic avian influenza (HPAI) occurrences, was constructed by extracting relevant information from the EMPRES-i database spanning the period from January 5, 2015, to March 7, 2023. This dataset encompasses comprehensive details pertaining to H5N1 and H5Nx subtypes, including the family of birds involved in transmission, observation and reporting dates, precise location coordinates, and the respective countries where the reports originated. The dataset comprises a total of 17,410 H5Nx and 8,383 H5N1 reported cases.

Environmental data acquisition

Previous literature reviews (Gilbert and Pfeiffer, 2012; Dhingra et al., 2016) have summarised the predictor variables commonly linked to occurrences of HPAI. Here, we explored four sets of environmental variables considered by Dhingra and colleagues (Dhingra *et al*. 2016), and which are detailed in the table in Supplementary Information Resources S1: host variables (set 1), land cover variables (set 2), eco-climatic variables (set 3), and a risk-based selection of variables performed by Dhingra and colleagues (set 4).

Set 1 includes log10-transformed extensive and intensive chicken density (Gilbert et al., 2015), duck density (Robinson et al., 2014) and human population density from the Gridded Population of the World (Center For International Earth Science Information Network-CIESIN-Columbia University, 2017) database. The chicken density data includes GIS layers representing the global distribution of chickens, categorised into extensive and intensive systems. It uses the model published by Gilbert et al. (2018) applied to the Gridded Livestock of the World (GLW) version 3 chicken layers (Gilbert et al., 2022a). The global duck distribution data were computed using the GLW version 4 (Gilbert et al., 2022b). Set 2 includes land cover information, which was obtained from the global 1-km Consensus Land Cover database by (Tuanmu and Jetz, 2014) Tuanmu and Jetz (2014), and identifies different land cover categories by providing a percentage of

representation of each land cover category within raster cells of approximately one square kilometre (*http://www.earthenv.org/landcover.html*). Additionally, this dataset was supplemented by a layer about the distance of each spatial point to the open water. Set 3 encompasses seasonal and large-scale patterns of eco-climatic indices like daytime land surface temperature (day LST) and the normalised difference vegetation index (NDVI), as documented by Scharlemann and colleagues (Scharlemann et al., 2008). Finally, set 4 is a selection from the previous sets based on prior epidemiological knowledge (Gilbert and Pfeiffer, 2012; Dhingra *et al*. 2016).

Ecological niche modelling

The different ecological niche modelling analyses were performed with the boosted regression trees (BRT), available in the R package "dismo" (Hijmans et al., 2017). BRT is a machine learning approach that can be used to generate a collection of sequentially fitted regression trees optimising the predictive probability of occurrence given local environmental conditions (Elith *et al*. 2006) The predictive probability, interpreted as ecological suitability for local virus circulation, ranges from "0" (unsuitable environmental conditions) and "1" (highly suitable environmental conditions). BRT allows us to model complex non-linear relationships between the response and various predictor variables (Elith *et al*. 2008). It has also been demonstrated that the BRT approach had a superior predictive performance compared to alternative modelling approaches (Elith et al., 2006).

Overall, we performed ten replicated BRT analyses based on each of the seven datasets of occurrence records considered in our study: (i) H5Nx cases in wild birds < 2020 (01/01/2015 - 31/12/2019), (ii) H5N1 cases in wild birds > 2020 (01/01/2020 - 06/03/2023), (iii) H5Nx cases in wild birds > 2020 (01/01/2020 - 06/03/2023), (iv) H5Nx cases in domestic birds < 2020 (01/01/2015 - 31/12/2019), (v) H5N1 cases in domestic birds < 2020 (01/01/2015 - 31/12/2019), (vi) H5N1 cases in domestic birds > 2020 (01/01/2020 - 06/03/2023), and (vii) H5Nx cases in domestic birds > 2020 (01/01/2020 - 06/03/2023). Of note, there was not enough occurrence data to train ecological niche models on H5N1 cases in wild birds < 2020 (only 33 occurrence data from eight different countries — Bangladesh, Bulgaria, China, India, Kazakhstan, Nepal, Romania, and Russia — between the 01/01/2015 and 31/12/2019).

We ran BRT analyses using both occurrence and "pseudo-absence" data, the latter corresponding to randomly sampled absence points in the study area (Elith et al., 2006). To address the heterogeneity of AIV surveillance efforts and to avoid misclassifying low-surveillance areas as unsuitable for virus circulation, we trained the ecological niche models only considering countries in which five or more cases have been confirmed. For China and Russia, given the size of these countries, we however considered the administrative polygons of level 1 (admins-1; provinces and federal districts, respectively) instead of the country polygons for the selection of administrative entities with at least five confirmed cases to be included in the study area. In practice, this implied that the study area used to train our models were restricted to those countries/admins-1 and that we only sampled pseudo-absence points in such restricted study areas. In addition, the sampling pseudo-absence points was based on the human population distribution to further account for potential within country differences in surveillance intensity (Dhingra *et al*. 2016): the probability to sample a pseudo-absence from a given grid cell was proportional to the log-transformed human population density in that cell. The human population density raster was retrieved from the Gridded Population of the World (GPW) project, which was downscaled to a resolution of 5 arcminutes. As pointed out by Dhingra and colleagues, the absence of the target pathogen in a region may simply be due to the fact that the pathogen has not (yet) been introduced in that region (Dhingra *et al*. 2016). To limit the impact of pseudo-absences that would have been sampled in those regions, a minimum distance of 10 km and a maximum distance of 1,000 km to the nearest presence points were therefore introduced to discard all simulated pseudo-absence points falling outside that range (Phillips *et al*. 2009, Dhingra *et al*. 2016). While we initially

simulated ten times more pseudo-absence than presence points, we eventually randomly retained three times more pseudo-absence points than presence points in each country/admin-1 included in the study area.

Given that spatial data typically exhibits autocorrelation, employing a standard cross-validation procedure may result in an overestimation of the BRT model's accuracy (Randin *et al.* 2006). To circumvent this issue, we employed a spatial cross-validation procedure to select the optimal number of trees in each BRT model. Specifically, we tested and compared two distinct spatial cross-validation procedures: (i) the procedure introduced by Dhingra and colleagues and based on (five) reference presence points used to cluster the presence and pseudo-absence points in (five) folds according to their nearest distance to a reference point, and (ii) the procedure introduced by Valavi and colleagues and based on a block generation method (Valavi *et al.* 2019). In the first spatial cross-validation procedure, the five reference presence points were randomly selected among all presence points. If each of the five generated spatial folds did not gather at least 10% of the presence points, the random selection of reference points were re-initiated. To compare the performance of the three cross-validation procedures applied in this study (the standard procedure and the spatial procedures based on the reference points or blocks generation), we estimated the spatial sorting bias (SSB) metric (Hijmans, 2012) ranging from 0 and 1, an SSB near 0 and 1 indicating an extreme impact and the absence of an impact of spatial autocorrelation on the model training, respectively.

All BRT analyses were run and averaged over 10 cross-validated replicates, with a tree complexity set at 4, an initial number of trees set at 10, a learning rate of 0.01, a tolerance parameter set to 0.001, a step size of 5, and considering 5 spatial folds. We evaluated the inferences using the area under the receiver operating characteristic (ROC) curve, also simply referred to as "area under the curve" (AUC). We further used the AUC metric to assess the capacity of our models trained on occurrence data <2020 to predict >2020 distribution of occurrence data. To assess how each environmental factor contributed to the different BRT models, we computed their relative influence (RI) in those models and their response curves showing how ecological suitability varies with one specific factor. RI values were obtained by evaluating the number of times a specific environmental factor was selected for splitting a tree, weighted by the squared improvement to the model as a result of each split, averaged over all trees (Elith *et al.* 2008).

We explored the four sets of environmental variables assembled by Dhingra and colleagues (Dhingra *et al.* 2016) and described above in the "Environmental data acquisition" subsection. The BRT analyses presented here were eventually performed based on set 2 for wild bird cases and set 4 for domestic bird cases. This selection is aligned with the methodology of Dhingra and colleagues (2016), prioritising variables which demonstrated the best predictive accuracy and clear epidemiological significance to minimise the potential for coincidental correlations. The RI values obtained when analysing each set of environmental variables separately are all reported in a table in Supplementary Information Resources S2.

Bird diversity indices calculation

Diversity index calculations were performed using the HPAI dataset. Cases lacking information on the date of collection, bird family, and subtype were excluded from the analysis. The diversity of HPAI cases across bird families was quantified using two indices: the Shannon diversity index and the Simpson diversity index. The Shannon index accounts for evenness of bird families (Shannon, 1948), whereas the Simpson index provides a measure of dominance (Simpson, 1949), emphasising the most abundant families in the dataset. Simpson index goes between 0 and 1, where 1 represents infinite diversity and 0, no diversity. Calculations were done using two time points: before and after 2020, and for various data classifications: overall and annually, across all bird families and specifically marine families, as well as for all subtypes (HxNx) and those identified as H5N1 subtype, wild birds, and poultry. These indices were calculated using the R

package “vegan” (Oksanen et al., 2023): the dataset was first aggregated by year, HPAI subtype and bird family, and we then used the “diversity” function to compute the Shannon and Simpson indices for each year.

Supplementary figures

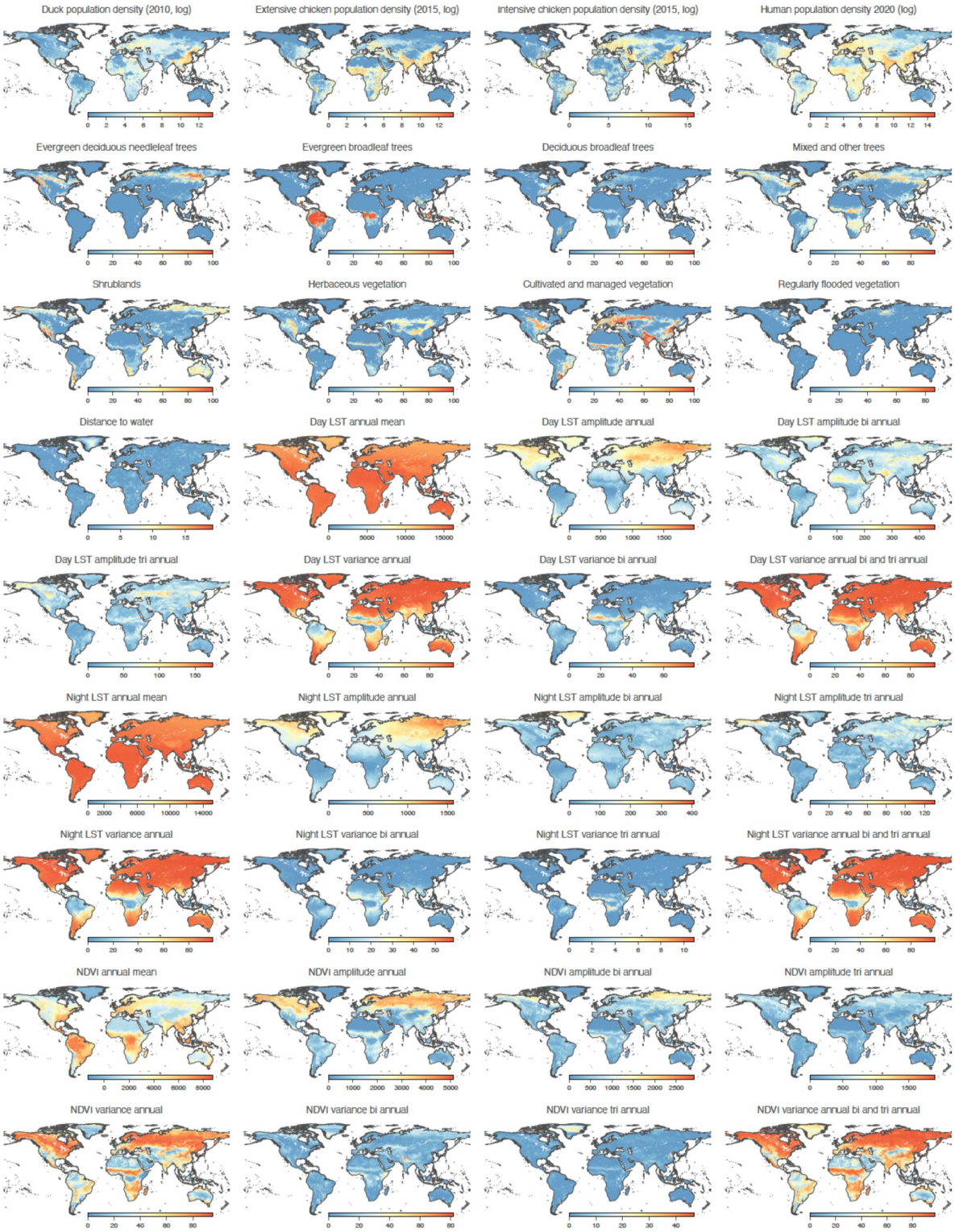

Figure S1 (part 1)

Environmental factors included in the ecological niche modelling.

"LST" refers to "land surface temperature", "NDVI" to the "normalised difference vegetation index", and "EVI" to the "enhanced vegetation index".

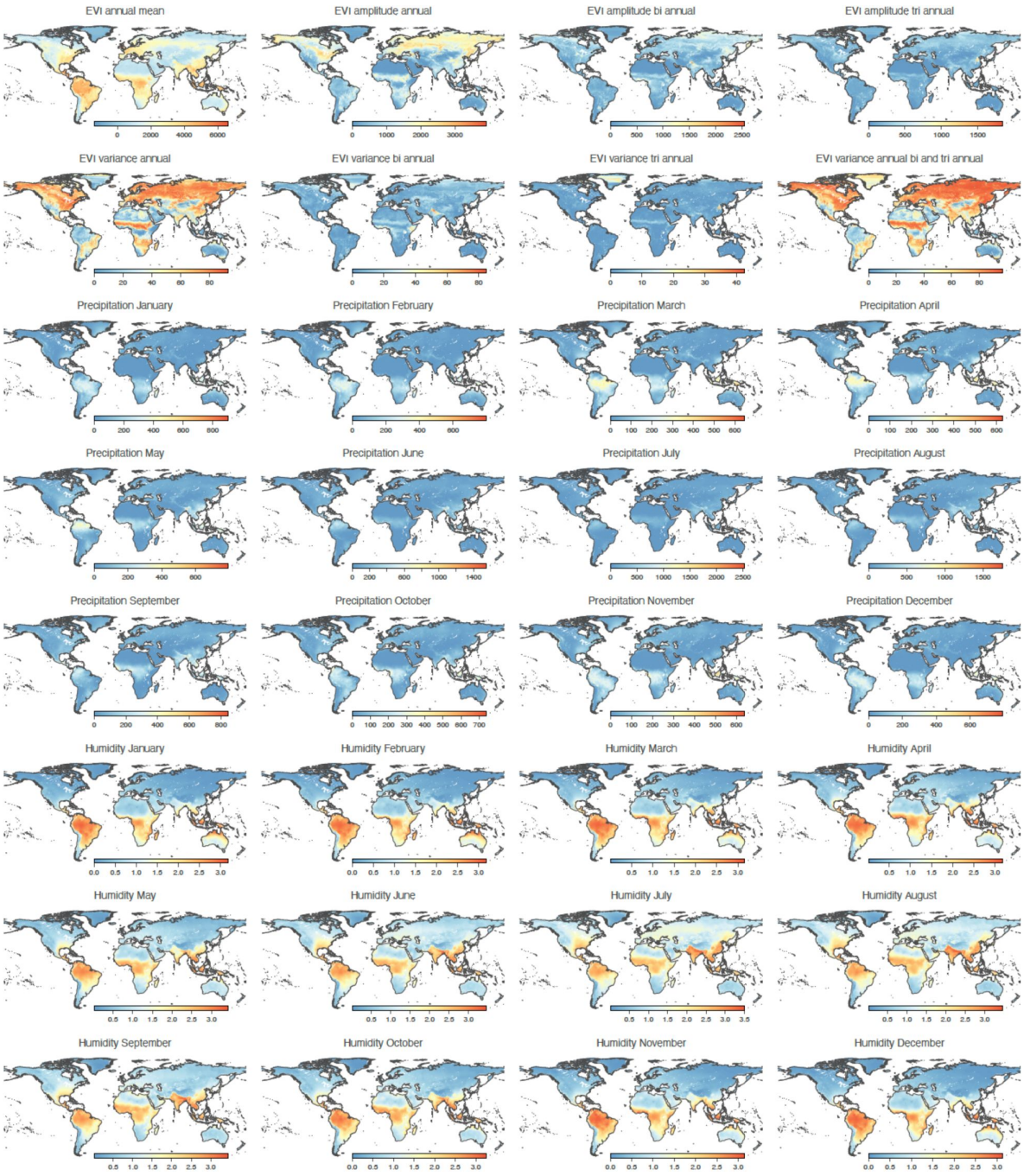

Figure S1 (part 2)

Environmental factors included in the ecological niche modelling.

"LST" refers to "land surface temperature", "NDVI" to the "normalised difference vegetation index", and "EVI" to the "enhanced vegetation index".

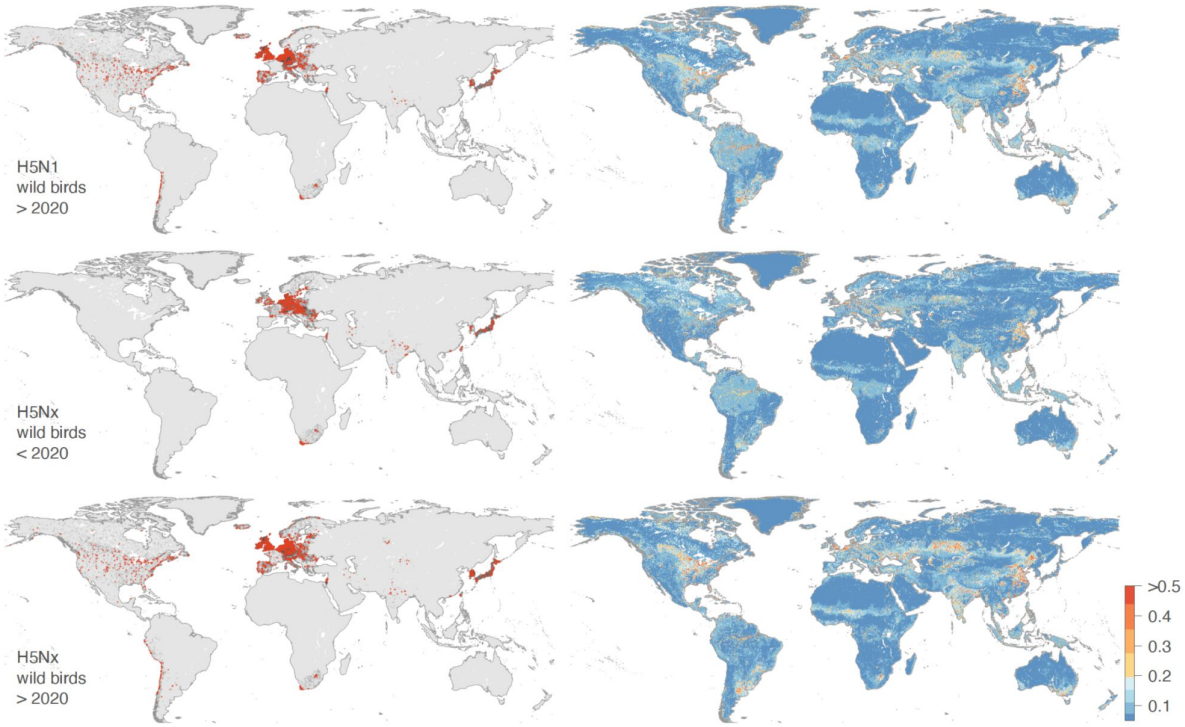

Figure S2 (part 1)

Comparison between the presence/pseudo-absence data used to train the ecological niche models (1° column) and the resulting ecological niche suitability maps (2° column).

In the first column, presence and sampled pseudo-absence points are displayed as red and dark points, respectively. As detailed in the Materials and Methods section, pseudo-absence points were only sampled in countries in which there was at least one presence point (for either H5N1 or H5Nx, the considering time period, and bird populations). For Russia and China, we considered the admin-1 administrative areas instead of the country to define if pseudo-absence points should be sampled in the corresponding area. Specifically, for a given administrative region (i.e. a given country or admin-1 region in case of Russia or China) gathering at least one presence point, we sampled a number of pseudo-absence points equal to three times the number of presence points in that region. Furthermore, pseudo-absence points were sampled according to the log-transformed raster of human population density, which prevented the sampling of pseudo-absence in unpopulated areas non associated with any surveillance activity.

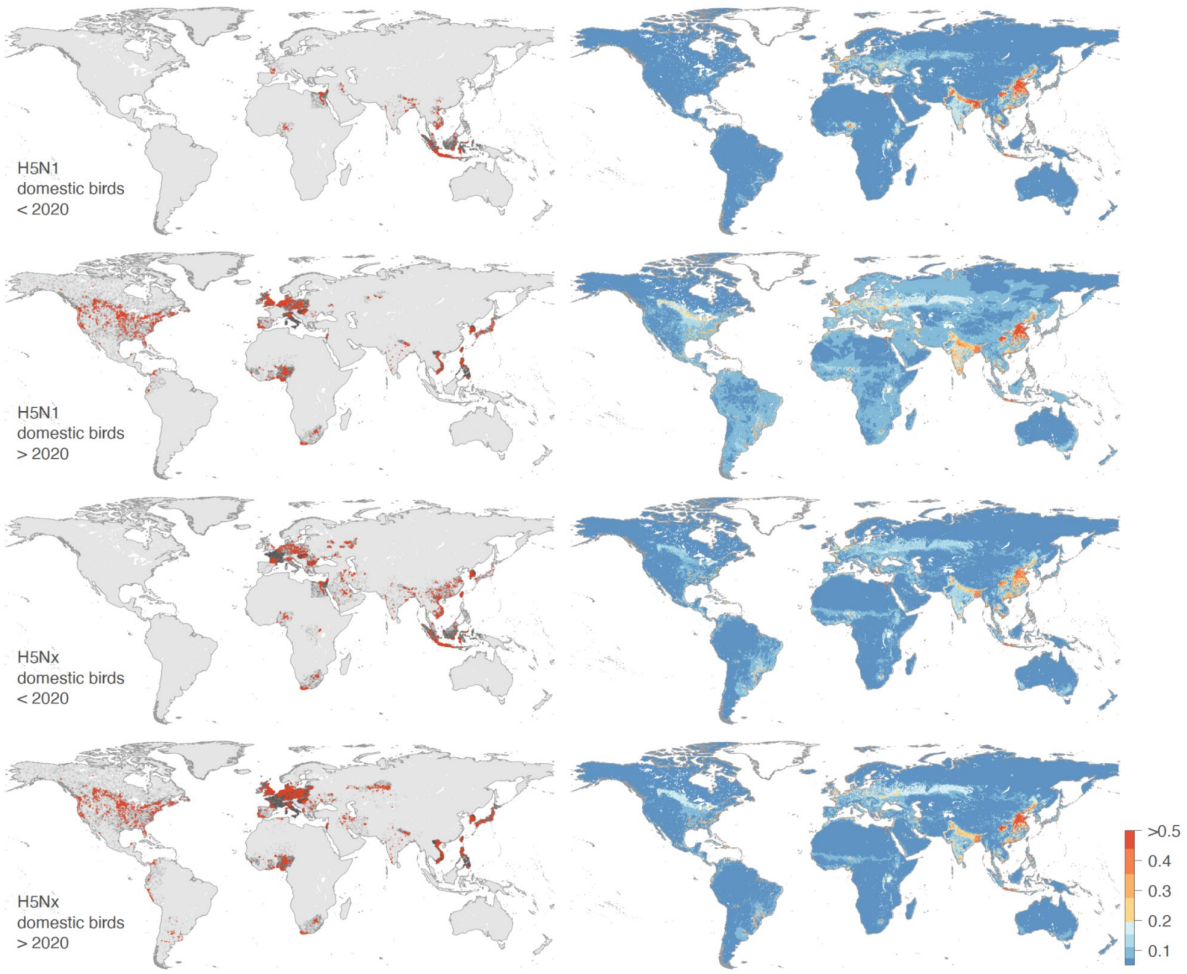

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Figure S3

Boxplots reporting the sorting sampling bias (SSB) and area under the curve (AUC) of the receiving operator curve metrics associated with various ecological niche models trained for H5N1 and H5Nx.

For the SSB estimates, we report values obtained for the standard cross-validation (A), as well as spatial cross-validation procedures based on the reference points (B) and the blocks generation (C) approaches (see the Materials and Methods section for further detail). SSB estimates range from 0 to 1; values close to 0 indicate a strong impact of spatial autocorrelation on model training, while values close to 1 suggest little to no impact. The AUC metrics computed for the ecological niche models trained with the spatial cross-validation procedures based on the "epicentres", and we here report the values obtained for the models trained on each of the four distinct sets of environmental factors.

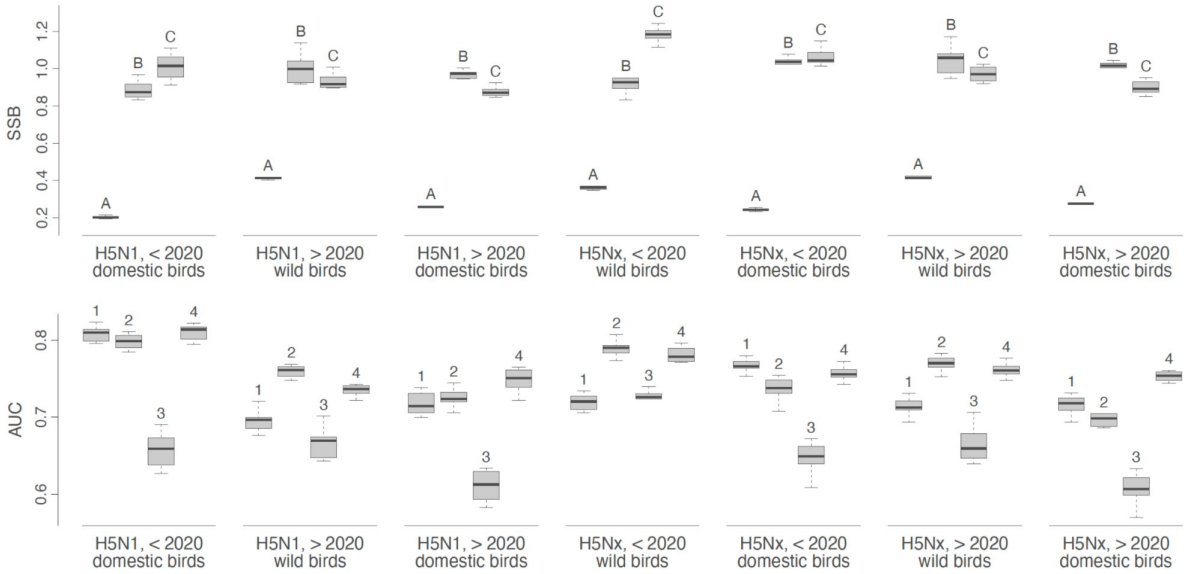
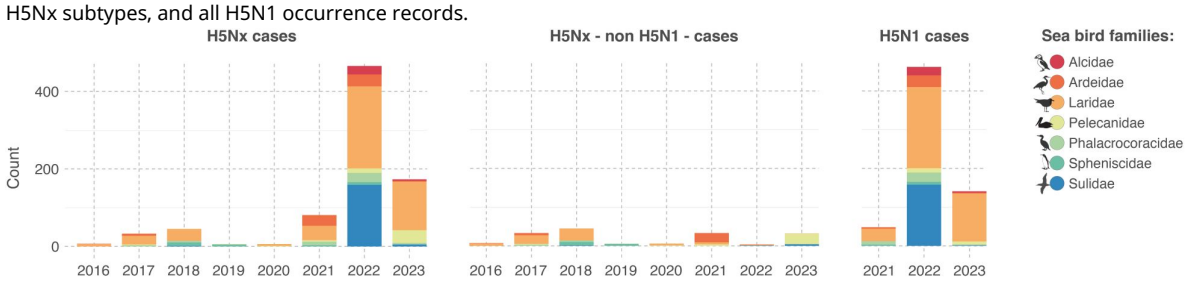


Figure S4

Distribution of H5Nx and H5N1 occurrence records in sea birds from 2015 to 2023, categorised by bird family.

The three panels successively show the total occurrence records for all H5Nx subtypes, occurrence records for non-H5N1 H5Nx subtypes, and all H5N1 occurrence records.



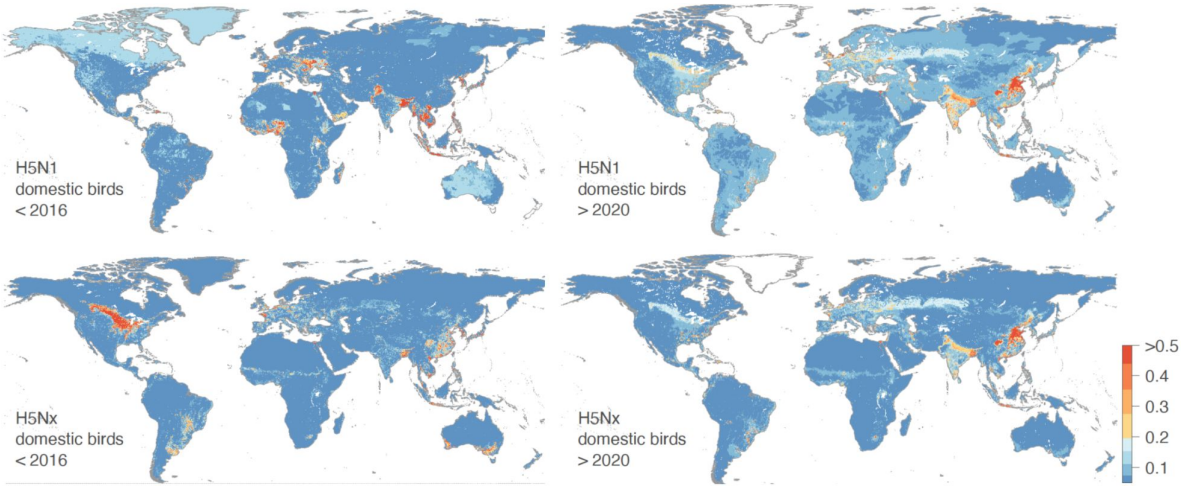


Figure S5

Comparison between the ecological niche suitability estimated for H5N1 and H5Nx before 2016 and after 2020 for domestic bird populations.

Specifically, we here compare the ecological niche models trained by Dhingra *et al.* (2016) on occurrence records collected between January 2004 to March 2015 (< 2016) and the ecological niche models trained in the present study on occurrence records collected between January 2020 to March 2023 (> 2020).

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Addtional information

Data and code accessibility

R scripts and related files needed to run the analyses, as well as Supplementary Information Resources S1 (description and source of each environmental variable included in the original sets of variables) and S2 (mean relative influence computed for the environmental variables within each considered set of variables), are all available at *https://github.com/sdellicour/h5nx_risk_mapping*.

Table S1

Assessment of the predictive performance of ecological niche models to predict present and past ecological suitability maps.

We here report, for each replicate analysis, a measure of the predictive performance of the trained ecological niche through the computation of the area under the receiver operating characteristic (ROC) curve, also simply referred to as "area under the curve" (AUC). (*) refers to AUC estimates obtained when assessing the capacity of models trained on occurrence data < 2020 to predict > 2020 distribution of occurrence data.

	Wild birds			Domestic birds					
	H5Nx			H5N1			H5Nx		
	< 2020	> 2020	>2020*	< 2020	> 2020	> 2020*	< 2020	> 2020	> 2020*
Replicate 1	0.899	0.793	0.700	0.832	0.812	0.769	0.833	0.791	0.755
Replicate 2	0.896	0.769	0.703	0.832	0.822	0.769	0.829	0.781	0.752
Replicate 3	0.895	0.796	0.707	0.834	0.820	0.770	0.818	0.784	0.743
Replicate 4	0.894	0.773	0.705	0.843	0.820	0.769	0.828	0.804	0.751
Replicate 5	0.893	0.797	0.706	0.837	0.816	0.770	0.828	0.780	0.752
Replicate 6	0.898	0.797	0.701	0.845	0.816	0.770	0.829	0.780	0.752
Replicate 7	0.897	0.798	0.700	0.835	0.816	0.769	0.827	0.775	0.750
Replicate 8	0.894	0.805	0.705	0.836	0.825	0.770	0.828	0.777	0.752
Replicate 9	0.894	0.770	0.702	0.834	0.813	0.769	0.828	0.779	0.750
Replicate 10	0.894	0.775	0.703	0.835	0.823	0.769	0.832	0.795	0.754

Table S2

Relative influence (RI, in %) of each environmental variable in the respective ecological models trained on a specific dataset of occurrence data.

A RI estimate was obtained for each BRT replicate and we here report median as well as first and third quartile RI values.

	Wild birds			
	H5N1		H5Nx	
	< 2020	> 2020	< 2020	> 2020
Evergreen deciduous needleleaf trees	-	1.6 % [1.5-1.6]	1.6 % [1.3-1.7]	0.4 % [0.4-0.5]
Evergreen broadleaf trees	-	1.3 % [1.2-1.4]	0.1 % [0.1-0.1]	1.0 % [0.8-1.1]
Deciduous broadleaf trees	-	4.1 % [4.0-4.2]	1.6 % [1.5-1.8]	6.1 % [5.9-6.2]
Mixed and other trees	-	11.5 % [11.4-11.7]	5.3 % [4.9-5.4]	11.4 % [11.3-11.8]
Shrublands	-	2.3 % [2.0-2.3]	3.2 % [3.0-3.3]	2.4 % [2.1-2.7]
Herbaceous vegetation	-	8.8 % [8.8-9.0]	2.8 % [2.5-3.0]	9.5 % [9.5-9.6]
Cultivated and managed vegetation	-	4.4 % [4.3-4.5]	4.8 % [4.6-4.9]	5.8 % [5.4-6.1]
Regularly flooded vegetation	-	0.3 % [0.3-0.3]	0.1 % [0.1-0.1]	0.2 % [0.1-0.3]
Urban and built-up areas	-	39.3 % [39.0-39.9]	54.5 % [53.5-55.0]	37.5 % [36.5-38.2]
Open water areas	-	22.0 % [21.9-22.3]	25.5 % [25.3-25.8]	23 % [22.8-23.3]
Distance to water	-	4.1 % [3.9-4.3]	0.9 % [0.7-1.0]	2.8 % [2.4-3.1]
	Domestic birds			
	H5N1		H5Nx	
	< 2020	> 2020	< 2020	> 2020
Duck pop. density (2010, log)	18.2 % [17.8-18.4]	15.7 % [13.3-16.4]	28.7 % [28.4-28.8]	12.9 % [12.9-13.5]
Extensive chicken pop. density (2015, log)	4.4 % [4.2-4.5]	2.3 % [0.5-2.6]	3.9 % [3.7-4.0]	2.7 % [2.5-3.4]
Intensive chicken pop. density (2015, log)	8.5 % [8.4-8.7]	30.4 % [29.6-36.2]	29.6 % [29.3-30.1]	43.1 % [40.5-43.4]
Human pop. density (2020, log)	54.9 % [53.9-55.1]	20.7 % [20.4-21.6]	15.5 % [15.3-15.7]	14.9 % [14.8-15.2]
Cultivated and managed vegetation	9.5 % [9.3-9.7]	16.6 % [16.3-18.5]	7.1 % [7.0-7.2]	13.2 % [12.9-13.3]
Open water areas	0.5 % [0.5-0.6]	1.4 % [0.3-1.5]	0.0 % [0.0-0.0]	0.6 % [0.4-0.8]
Day LST annual mean	4.1 % [4.0-4.2]	12.9 % [9.6-13.3]	15.4 % [15.1-15.6]	13.0 % [12.4-13.9]

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Reviewer #1 (Public review):

Summary:

The authors aim to predict ecological suitability for the transmission of highly pathogenic avian influenza (HPAI) using ecological niche models. This class of models identify correlations between the locations of species or disease detections and the environment. These correlations are then used to predict habitat suitability (in this work, ecological

suitability for disease transmission) in locations where surveillance of the species or disease has not been conducted. The authors fit separate models for HPAI detections in wild birds and farmed birds, for two strains of HPAI (H5N1 and H5Nx) and for two time periods, pre- and post-2020. The authors also validate models fitted to disease occurrence data from pre-2020 using post-2020 occurrence data.

Strengths:

The authors follow the established methods of Dhingra et al., 2016 to provide an updated spatial assessment of HPAI transmission suitability for two time periods, pre- and post-2020. They explore further methods of model cross-validation and consider the diversity of the bird species that HPAI has been detected in.

Weaknesses:

The precise ecological niche that the authors are modelling here is ambiguous: if we treat the transmission of HPAI in the wild bird population and in poultry populations as separate transmission cycles, linked by spillover events, then these transmission cycles are likely to have fundamentally different ecological niches. While an "index case" in farmed poultry is relevant to the wildlife transmission cycle, further within-farm and farm-to-farm transmission is likely to be contingent on anthropogenic factors, rather than the environment. Similarly, we would expect "index cases" in outbreaks of HPAI in mammals to be relevant to transmission risk in wild birds - this data is not included in this manuscript. Such "index cases" in farmed poultry occur under separate ecological conditions to subsequent transmission in farmed poultry, so should be separated if possible. Some careful editing of the language used in the manuscript may elucidate some of my questions related to model conceptualisation.

The authors' handling of sampling bias in disease detection data in poultry is possibly inappropriate: one would expect the true spatial distribution of disease surveillance in poultry to be more closely correlated with poultry farming density, in contrast to human population density. This shortcoming in the modelling workflow possibly dilutes a key finding of the Results, that the transmission risk of HPAI in poultry is greatest in areas where poultry farming density is high.

https://doi.org/10.7554/eLife.104748.1.sa1

Reviewer #2 (Public review):

Summary:

This study aimed to determine which spatial factors (conceived broadly as environmental, agronomic and socio-economic) explain greater avian influenza case numbers reported since 2020 (2020--2022) by comparing similar models built with data from the period 2015--2020. The authors have chosen an environmental niche modelling approach, where detected infections are modelled as a function of spatial covariates extracted at the location of each case. These covariates are available over the entire world so that the predictions can be projected back to space in the form of a continuous map.

Strengths:

The authors use boosted regression trees as the main analytical tool, which always feature among the best-performing models for environmental niche models (also known as habitat suitability models). They run replicate sets of the analysis for each of their model targets (wild/domestic x pathogen variant), which can help produce stable predictions. The authors take steps to ameliorate some forms of expected bias in the detection of cases, such as

geographic variation in surveillance efforts, and in general more detections near areas of higher human population density.

Weaknesses:

The study is not altogether coherent with respect to time. Data sets for the response (N5H1 or N5Hx case data in domestic or wild birds) are divided into two periods; 2015--2020, and 2020--2022. Each set is modelled using a common suite of covariates that are not time-varying. That suggests that causation is inferred by virtue of cases being in different geographic areas in those two time periods. Furthermore, important predictors such as chicken density appear to be informed (in the areas of high risk) from census data from before 2010. The possibility for increased surveillance effort *through time* is overlooked, as is the possibility that previously high-burden locations may implement practice changes to reduce vulnerability.

https://doi.org/10.7554/eLife.104748.1.sa0

Author response:

> ***Reviewer #1:***
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> *Summary:*
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> *The authors aim to predict ecological suitability for the transmission of highly pathogenic avian influenza (HPAI) using ecological niche models. This class of models identify correlations between the locations of species or disease detections and the environment. These correlations are then used to predict habitat suitability (in this work, ecological suitability for disease transmission) in locations where surveillance of the species or disease has not been conducted. The authors fit separate models for HPAI detections in wild birds and farmed birds, for two strains of HPAI (H5N1 and H5Nx) and for two time periods, pre- and post-2020. The authors also validate models fitted to disease occurrence data from pre-2020 using post-2020 occurrence data.*
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We apologise if this aspect was not clear enough in the previous version of our manuscript but our analyses do not treat or make the assumption of distinct transmission cycles between wild and domestic bird species; those transmission cycles being indeed interconnected by frequent spillover events. Yet, we indeed conduct independent ecological niche modelling analyses to estimate both the ecological suitability for the risk of local circulation in domestic birds as well as the ecological suitability for the risk of local circulation in wild birds. This distinction does not imply that the virus circulates exclusively within one of these populations but rather allows us to identify potential differences in the environmental conditions associated with virus occurrences in each context.

Our results indicate that these two ecological niche models capture distinct environmental patterns. Virus occurrences in wild birds were primarily associated with factors such as open water and proximity to urban areas, while occurrences in domestic birds were more strongly linked to variables like poultry density and cultivated vegetation. This finding supports the existence of two distinct ecological niches for the virus, corresponding to virus circulation in wild and domestic bird populations. We thank the Reviewer for their feedback and we will take this opportunity to further clarify this aspect in the text.

> *While an "index case" in farmed poultry is relevant to the wildlife transmission cycle, further within-farm and farm-to-farm transmission is likely to be contingent on anthropogenic factors, rather than the environment. Similarly, we would expect "index cases" in outbreaks of HPAI in mammals to be relevant to transmission risk in wild birds - this data is not included in this manuscript. Such "index cases" in farmed poultry occur under separate ecological conditions to subsequent transmission in farmed poultry, so should be separated if possible. Some careful editing of the language used in the manuscript may elucidate some of my questions related to model conceptualisation.*

We agree, but index cases are particularly difficult to separate from secondary spread in the absence of field investigation. Identification of index cases based on space-time filtering have been previously investigated but are strongly dependent on the quality of the surveillance, i.e. an “apparent” primary case can be a secondary case of previously undetected ones, and constant surveillance quality cannot be assumed to be homogeneous across countries. Our ecological niche modelling approach is based on HPAI cases reported in the EMPRES-i database, which includes all documented outbreaks without distinguishing primary introductions from subsequent farm-to-farm transmissions. Thus, our ecological niche models are trained on confirmed cases that result from a combination of different transmission dynamics, including introduction events in poultry populations (which can be impacted by ecological factors) and persistence within and between poultry populations (which can be impacted by anthropogenic factors).

For clarity, we will revise the manuscript to clarify that, while our study primarily aims to assess the environmental suitability for HPAI occurrences, the dataset does not exclude cases resulting from farm-to-farm spread. This means that our models can capture the environmental variables associated with the risk of cases associated with both primary introductions (e.g., spillover from wild birds) and secondary transmission events within poultry systems, although the latter is also influenced by anthropogenic factors such as biosecurity practices and poultry trade networks. These latter factors are not included in our models, which will be highlighted in the limitations (Discussion section) of the revised manuscript.

In addition, we note the Reviewer's comment regarding the relevance of “index cases” in mammalian outbreaks to understanding the risk of HPAI transmission in wild birds. Although these data are not included in our current study, we will highlight the potential value of incorporating these cases into future models in order to refine risk predictions, provided that they can be identified with some reasonable level of certainty.

> *The authors' handling of sampling bias in disease detection data in poultry is possibly inappropriate: one would expect the true spatial distribution of disease surveillance in poultry to be more closely correlated with poultry farming density, in contrast to human population density. This shortcoming in the modelling workflow possibly dilutes a key finding of the Results, that the transmission risk of HPAI in poultry is greatest in areas where poultry farming density is high.*

The Reviewer raises a valid point that poultry surveillance efforts can also be considered as correlated with poultry farm density than with human population density. While human population density can serve as a reasonable proxy for surveillance intensity — given that disease detection is often more active in areas with stronger veterinary notification systems — we acknowledge that poultry disease surveillance can also be influenced by the spatial distribution of poultry farms, as high-density poultry areas could be prioritised for monitoring. Please note that in our study, we followed a previously established approach (Dhingra et al. 2016) and weighted pseudo-absence sampling based on human population density to account for general surveillance biases. However, we do not agree with the Reviewer's point. In fact, assuming a sampling bias correlated with poultry density would result in reducing its effect as a risk factor. The current approach does not.

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We acknowledge the Reviewer's comments regarding the consistency of time periods in our study. Our approach is to divide the HPAI case data into two time periods (2015-2020 and 2020-2022) and ecological niche models using a common set of covariates that do not explicitly account for temporal variation. We will further clarify these aspects in the revised version of our manuscript:

(1) Our primary objective is to assess changes in ecological suitability over time rather than infer direct causation. By comparing models trained on pre-2020 data with post-2020 occurrences, we evaluate whether pre-2020 environmental conditions can predict recent

HPAI suitability. However, we acknowledge that this does not capture dynamic changes in surveillance efforts, biosecurity measures, or host-pathogen interactions over time.

(2) Regarding predictor variables, we used poultry density data from 2015, rather than pre-2010 data. However, this dataset is not based on a single census year; instead, it represents a median estimate derived from subnational poultry census data collected between 2000 and 2019. This median year approach provides a more stable representation of poultry density than any single-year snapshot. Furthermore, while poultry production systems may exhibit some temporal variation, these changes are generally minor compared to the inter-annual variability observed in HPAI occurrence, which is largely driven by epidemic dynamics. Given the current limitations of global poultry data, distinguishing distributions from different years is not feasible with the available GLW dataset. We will clarify these points in the manuscript.

(3) We recognise that increased surveillance efforts and adaptive changes in poultry farming practices could influence the observed HPAI case distribution. While our current models do not incorporate time-varying surveillance intensity or biosecurity policies, we will address this limitation in the Discussion section and suggest that future work integrates dynamic surveillance data to improve risk assessments.

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